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14. ABSTRACT The long-term goal of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin they represent an in vivo test bed to examine both tumor biology and the application of therapeutic agents. Over the lifetime of this grant we have developed a new metastatic model based upon overexpression of c-Myc in primary cultures of human prostatic epithelial cells. This model was extremely aggressive and we therefore altered the insult from high overexpression of c-Myc towards a more moderate suppression of PTEN. This resulted in a premalignant phenotype which we continue to explore. Concurrently we have developed two new human prostatic epithelial cell lines which, in tissue recombinants behave like normal prostatic epithelia but are able to act as recipients for virally transduced genetic insults providing a basis for new cancer models and a resource for the community. These have been distributed and a descriptive paper submitted. We also developed a new orthotopic model of prostate cancer metastasis.

15. SUBJECT TERMS
tissue, recombination, prostate cancer, in vivo, metastasis, c-Myc, oncogene, model

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Introduction

The **long-term goal** of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin they represent an in vivo testbed to examine both tumor biology and the application of therapeutic agents.

As proposed we have generated and used models in which human prostatic epithelial cells (huPrE) are grown in tissue recombinants with rat urogenital sinus mesenchyme (rUGM) and grafted back into the in vivo environment of an intact male athymic rat host. Manipulations of the huPrE allow us to examine the effects of retroviral transfection with c-Myc within the huPrE. Our original C7-Myc model forms aggressive tumors which move rapidly from a benign to metastatic phenotype. Hence, we have generated new molecular and cellular tools and from these have made less aggressive models (as originally proposed), which allow us to follow the progressive events in cancer initiation and progression.

Work Completed

Over the lifetime of the grant we achieved a number of important aims. The first of these was establishing and testing the C7-Myc model – which was the original basis of the proposal. This model was described in a paper published in 2005:

Williams, K., Fernandez, S., Stien, X., Ishii, K., Love, H.D., Lau, Y-F., Roberts, R.L., Hayward, S.W. [2005] Unopposed c-MYC expression in benign prostatic epithelium causes a cancer phenotype. Prostate 63, 369-384

This work revealed that the approach we were taking was powerful but had some drawbacks. In particular the use of a cmv-Myc construct was clearly too strong of a stimulus. In addition it was clear that metastasis from the renal capsule site did not mimic the pattern seen in human prostate cancer patients. Third it was considered that having human prostatic epithelial cell lines which responded like normal cells but which were susceptible to viral manipulation would be a more reliable basis for future work than the use of primary cultures, with their inherent patient to patient differences.

As described in previous annual reports to address the issue of metastatic spread profile we examined the possibility of using the prostatic orthotopic grafting site to assess this aspect of tumorigenesis. For this work we used the PC3 cell line which is known to have metastatic potential. We developed an intraductal grafting system whereby cells were grafted directly into the ducts of the mouse prostate. As shown in figures 1 and 2 (below) this work demonstrated that the prostatic orthotopic site was a superior venue for investigation of metastatic activity versus the renal capsule.

Modeling of spontaneous advanced human prostate cancer metastasis in a new mouse model.

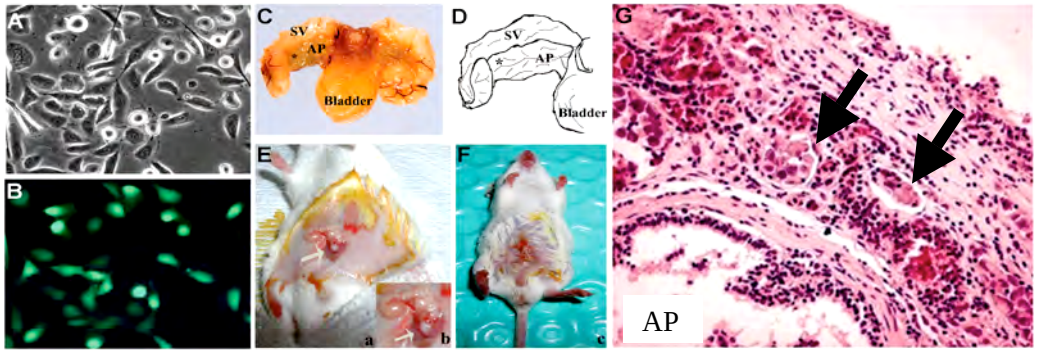

Figure 1. An intraductal anterior prostate (AP) orthotopic xenografting SCID mouse model.

Figure 1. PC-3-EGFP cells grow in RPMI 1640 plus 5% FBS under the phase-contrast (**A**) and the GFP-fluorescence microscope (**B**). The intraductal anterior prostate (AP) orthotopic xenografting mouse model. Schematic showing an incision in a mouse main duct of AP (**C, *** and **D, ***). A graft is placed inside an AP duct (**E, a,** low magnification and **b**, high magnification, arrow). The abdominal wound is sutured (**F**). Emboli of PC-3-EGFP cells, confirmed by anti-EGFP IHC staining, are shown in the microvessels under the mouse prostatic capsule (**G**, arrow).

Multi-organ metastasis including musculoskeletal tissues by orthotopic xenografting of PC-3-EGFP cells.

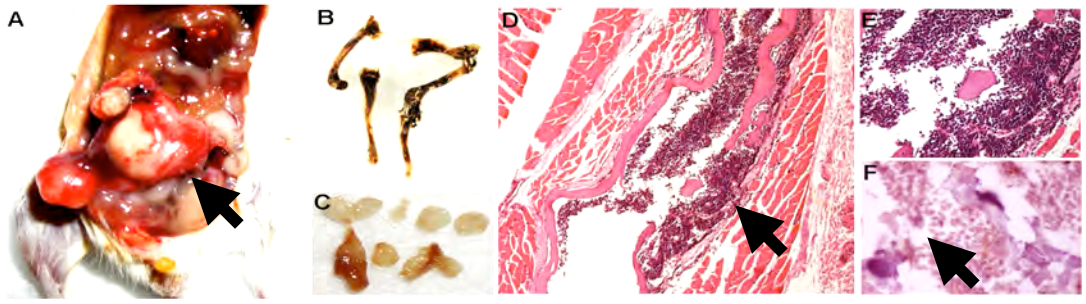

Figure 2. Osteolytic pathogenesis in mouse femur induced by the metastatic PC-3-EGFP cells.

Figure 2. PC-3 tumor grows in the primary grafted site post-grafting at 7 wks (**A**, arrow) and invades to the ipsilateral seminal vesicle. Dissected mouse hind limbs, femur and tibia are shown (**B**). Dissected enlarged metastatic lymph nodes are presented (**C**). Histology shows the osteolytic pathogenesis in a mouse femur (**D**, arrow, Low magnification and **E,** High magnification). An anti-EGFP immunohistochemical staining is used for tracing metastatic PC-3 cells mixed with bone marrow cells (**F**, arrow).

This approach allows tumors to form in a manner analogous to that seen in human patients. Of particular significance, grafts to this site metastasize in a pattern similar to that seen in human patients. The tumor cells migrate along the spinal column and invade the spine and major bones as well as the liver, lungs and other organs. This is important because metastatic spread to the bone is an important biological component of human prostate cancer which has not been easy to model in the in vivo systems used

historically. A manuscript describing this aspect of the project is currently being written up for publication.

To address the issue of consistency between epithelial cells we developed new human prostatic epithelial cell lines (NHPrE and BHPrE) which are able to replicate many critical aspects of human prostate including expression of both androgen receptors and PSA illustrated in figure 3 (below). A manuscript describing the NHPrE and BHPrE lines has been submitted for publication.

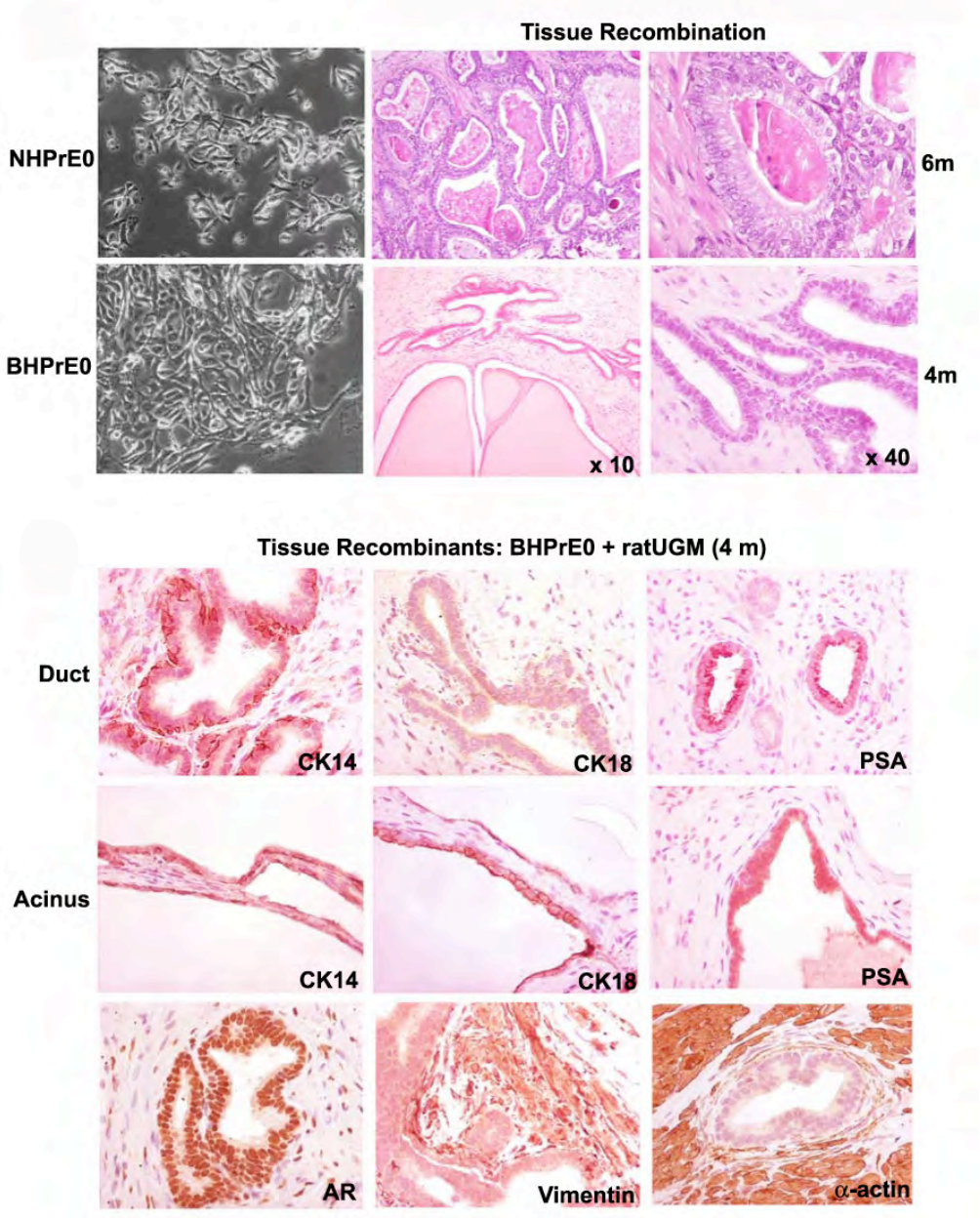

Figure 3. NHPrE and BHPrE cells recombined with rat urogenital sinus mesenchyme. The two cell lines both form glandular structures which show appropriate stromal and epithelial organization and marker expression.

Jiang, M., Fernandez, S., He, Y., Yi, Y., Birbach, A., Yuan, J., Williams, K., Protopopov, A., Chin, L., Susan Kasper, S., Tang, D.G. and Hayward, S. W. Functional remodeling of benign human prostatic tissues *in vivo* by definitive progenitor and intermediate cells (submitted)

In order to investigate whether overexpression of c-Myc resulted in carcinogenesis in these cells we introduced the C7-Myc construct and generated tissue recombinants using rUGM.

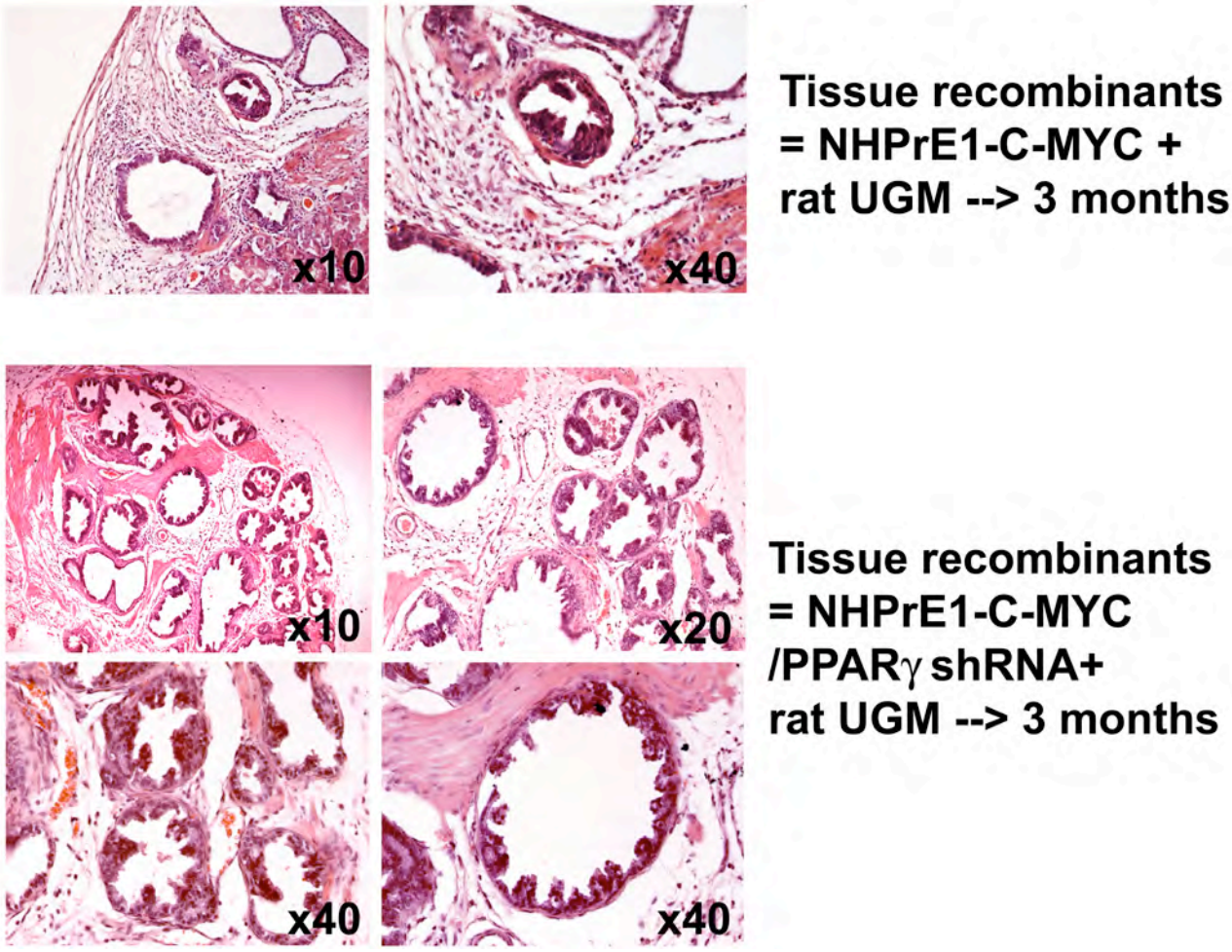

Figure 4. Consequences of c-Myc overexpression and PPARγ suppression on the differentiation of NHPrE +rUGM recombinants. In the upper panel it is evident that overexpression of c-Myc in the epithelial cells resulted in the formation of preneoplastic PIN lesions. In the lower panel additional suppression of PPARγ enhanced the frequency and severity of this effect.

When c-Myc was overexpressed in the NHPrE cells no evidence of malignant progression was seen over a three month experimental period. In accordance with the concepts outlined in specific aim 3 we

therefore added additional genetic insults to the model. In the lower panel of figure 4 the suppression of PPARγ signaling in the c-Myc overexpressing epithelial cells resulted in a more severe and widespread PIN phenotype than overexpression of c-Myc alone. Loss of PPARγ signaling due to loss of enzymes making the ligands for this nuclear receptor is a common occurrence in early human prostate cancer.

Suppression of PTEN expression (common in human prostate cancer) has also been tested in this model. The results of this experiment show that suppression of PTEN results in a preneoplastic (PIN) phenotype, as shown in figure 5.

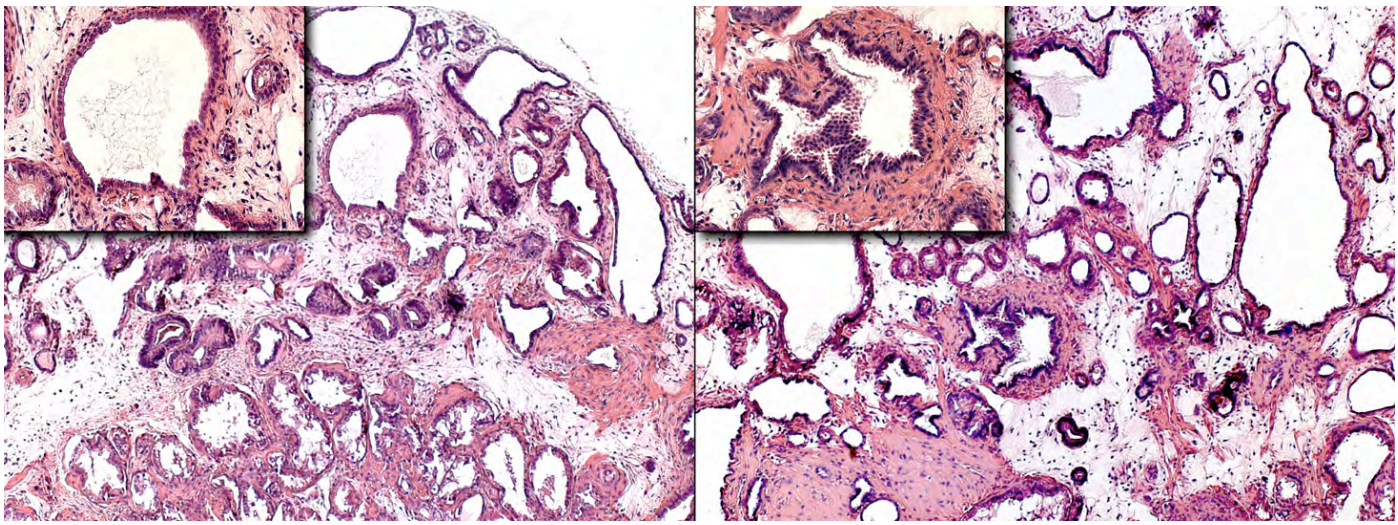

Figure 5. Control shRNA (left panel) and PTENshRNA (right panel) expressed in NHPrE cells which were then selected and recombined with rUGM for three months. Control grafts resemble those shown in figure 3. In contrast suppression of PTEN expression resulted in epithelial piling and cytologic changes consistent with PIN.

In additional related experiments we have been able to show, in a collaboration with the Bhowmick laboratory, that expression of c-Myc in prostatic stromal cells results in phenotypic changes consistent with those seen in human prostatic carcinoma associated fibroblasts (CAF). This adds to data, some published and some not, showing that the CAF phenotype is complex and can result from a number of different genetic and phenotypic changes.

In an effort to pursue data on the role of gene expression in the genesis of prostate cancer we have also been (somewhat peripherally) involved in a project with the Matusik laboratory which has resulted in the generation of a new mouse model of prostate cancer based upon the downregulation of the cell cycle

control regulator p57Kip2 which results in a prostatic phenotype which closely resembles human prostatic carcinogenesis.

Jin, R.J., Lho, Y., Wang, Y., Ao, M., Revelo, M.P., Hayward, S.W., Wills, M.L., Logan, S.K., Zhang, P. and Matusik, R.J. [2008] Down regulation of p57Kip2 induces prostate cancer in the mouse *Cancer Research* **68**, 3601-3608

Technical Modifications

As described in previous reports we have modified the orthotopic site graft method to use intraductal grafting which has proven to give a more reliable pattern of metastatic spread, more closely resembling that seen in human prostate cancer patients. We have also developed and incorporated human prostatic epithelial cell lines which were not available at the time this proposal was written. These allow more consistent data to be generated than the proposed use of primary epithelial cultures. Further we have generated new models which show a more measured development of malignancy than that seen in previous models and have developed tools to selectively modify this progression tetracycline regulation of shRNA expression.

Personnel Changes

None since the last report

Key Research Accomplishments

- *Development of a new model of prostate cancer metastasis based upon orthotopic intraductal xenografting.*
- *Generation and in vivo testing of two new benign human prostatic cell lines which recapitulate normal prostatic developmental milestones including expression of androgen receptor and PSA and which can serve as a basis for further model development based upon specific genetic changes.*
- *Characterization of viral vectors to suppress PTEN expression and to activate Akt signaling in tissue recombinants using human prostatic epithelium and rUGM.*
- *Establishment of new cells lines (based on the normal lines described above) overexpressing c-Myc both alone and in combination with suppression of PPARγ. These models provide a much more measured progression to malignancy than the original C7-Myc line. This work also*

demonstrates that two common early changes in human prostate cancer can act additively to induce a phenotypic response.

- *Development of tet-regulated PTEN suppression models.*

Reportable Outcomes.

Two papers published listed above. Further publications in process.

Conclusions.

This project has generated a number of important new reagents, model systems and techniques. Notable amongst these are the development of a new orthotopic metastasis model. This will be useful for many future studies. We have also generated new cell lines which will expand the repertoire available to the research community. These have been shown to recapitulate normal prostatic development in vivo (a characteristic not seen in other cell lines). These cells have been used as a basis for new models and have shown their ability to act as specific recipients for virally-introduced genetic insults. As such these cells will be of great utility to the community, and have already been requested by a number of laboratories working in the prostate cancer field (we anticipate wide demand once the descriptive manuscript is published).

We have demonstrated the feasibility of introducing oncogenes or suppressing expression of tumor suppressor genes to generate in vivo models of various stages of human prostate cancer. This work provides a series of novel and biologically relevant in vivo models with which to continue exploring the role of genetic changes of human prostatic epithelium in prostate cancer.